

# **Planar Laser Imaging of Scattering and Fluorescence of Zooplankton Feeding in Layers of Phytoplankton *in situ***

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## **LONG-TERM GOALS**

We intend to quantify the biological, physical, and chemical dynamics that structure marine planktonic ecosystems. Observations of the organisms and their environment on the spatial and temporal scales that characterize their interactions, combined with models of the dominant dynamics, will lead to improved understanding of the dynamics, structure, and function of planktonic ecosystems.

## **OBJECTIVES**

Our objectives in this work are to 1) visualize and quantify herbivorous copepod feeding in the laboratory, and 2) to apply these methods in the field to observe the dynamics of copepod feeding *in situ*. In particular we intend to test the “feeding sorties” hypothesis vs. the “*in situ* feeding” hypothesis regarding the location and timing of copepod feeding and vertical migration.

## **APPROACH**

Previous attempts to quantify copepod feeding have either been indirect (measuring the phytoplankton concentration before and after copepods were introduced to a sample), or direct (measuring the gut fluorescence of individual copepods feeding on phytoplankton). The disadvantage of the first method is that we obtain little information about the activities of individual copepods, and how their feeding might change in time. The second method is destructive, and generates only one data point per individual copepod, rendering it ineffective for generating time series of feeding activity. To obviate these problems, we use a planar laser imaging fluorometer (PLIF) system for quantifying copepod gut fluorescence and feeding. A green (532 nm) laser is used to stimulate the fluorescence of chlorophyll *a* ingested by copepods. The fluoresced red (680 nm) light is imaged by a very sensitive CCD camera. We also designed and built a second bi-spectral PLIF system that images both the fluorescence from chlorophyll *a*, and the green light scattered from particles (including copepods) in the imaging plane.

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This method is non-destructive, allowing time-series measurements to be made on individual copepods.

To image copepods in the field, we constructed a two-camera system, that would take images in synchrony. One camera was equipped with a 530 nm filter to detect the shape of imaged particles; the second camera was equipped with a 685 nm filter to image fluorescent particles; both cameras imaged the same plane. The constraints imposed by the system's geometry limited our image resolution to about 80x80 microns on an imaging plane of 10 x 13 cm, adequate for our purpose. By profiling vertically with this bi-spectral imaging system we can identify copepods, determine the depth at which they are located, and determine whether they have recently fed.

Our tasks for the third year of our research were to:

- 1) Complete the analysis of the copepod feeding experiments.
- 2) Complete the analysis of the data from Dabob Bay cruise.

## WORK COMPLETED

We have had great success in achieving our goals in this second part of our research. The experiments performed in the lab generated a large set of unique data documenting the gut activity of individual copepods. Using this data set, we are currently developing new insights on how a copepod's gut works and how that might relate to its feeding behavior.

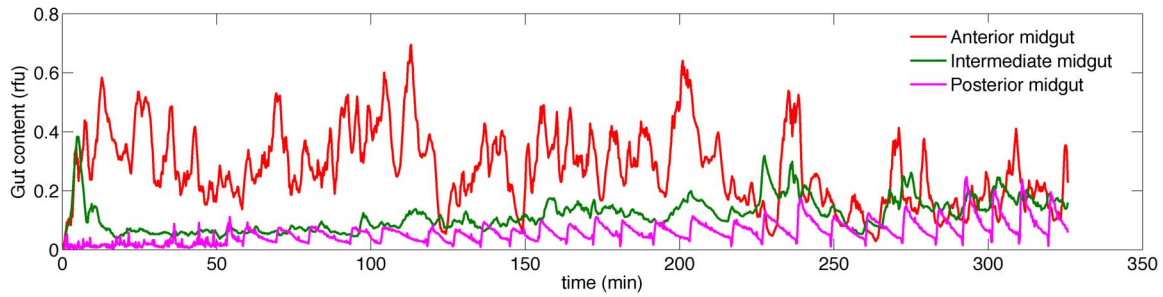
We deployed the *in situ* bi-spectral imaging system during a cruise on the R/V Thompson in May of 2007, in conjunction with Dr Bruce Frost's sampling work. In combination with the Frost data and our ancillary measurements, the optical data set we acquired will allow us to test the "feeding sorties" hypothesis against the "*in-situ* feeding" hypothesis.

A manuscript describing the methodology used in the lab feeding experiments has been accepted to *Limnology and Oceanography Methods*.

## RESULTS

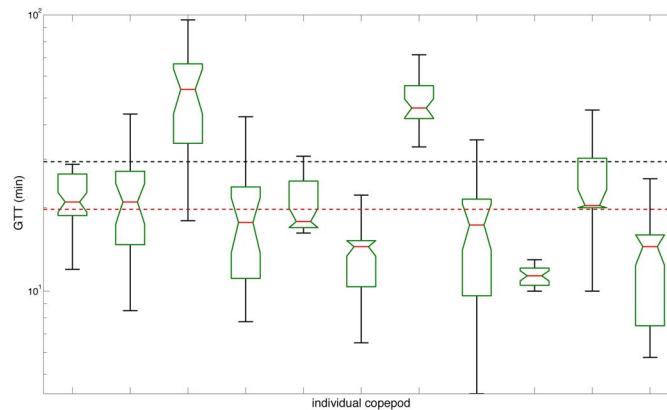
Our laboratory imaging of the feeding of tethered copepods shows the mid-gut of *C. pacificus* is functionally divided into three compartments: the anterior mid-gut (AMG), the intermediate mid-gut (IMG), and the posterior mid-gut (PMG). During feeding food enters the AMG where it forms a bolus. Portions of this bolus can move back and forth between the AMG and the IMG. After the IMG the semi-digested food moves to the PMG where the fecal pellet is formed.

The time series of chlorophyll *a* fluorescence in the three different gut compartments (Fig. 1) are quite revealing of the short time scale (minutes) and long time scale (hours) fluctuations in feeding, digestion and egestion. With images taken every 10 seconds, it is clear that the copepods have both long-term rhythms in their feeding (~40 minute fluctuations) as well as shorter time scale variability (5-10 minutes). A particularly striking feature of the copepod's gut dynamics is the very regular pulsing of the PMG, quite independent of the AMG and IMG. The period of this pulsing is temperature-dependent, with faster pulses in higher temperatures.



**Fig. 1. Time series of chlorophyll a fluorescence from three gut compartments of a tethered copepod. Images are acquired every 10 s. Note the very regular pulsing of the PMG, in contrast to the more irregular rhythms of the AMG and IMG.**

One measure of copepod feeding is gut throughput time (GTT). In previous work GTT has been estimated by measuring the time it takes a fed copepod to empty its gut when placed in filtered (food-free) seawater. This technique suffers from two limitations: first only a single data point is obtained for each copepod, and second, it is assumed that GTT for a copepod in food-free water is the same as a feeding copepod. It is also assumed that the average GTT obtained from many copepods would be representative of a population's grazing activity. We have used our data to calculate GTT time series from dozens of individual feeding copepods. The GTT show that differences in GTT among individuals are significant, suggesting that an overall average GTT may not be very representative (Fig. 2). We also found that GTT was significantly different in feeding vs. non-feeding copepods; GTT was shorter then copepods were feeding. We conclude that these differences in gut dynamics suggest that previous results obtained from full but non-feeding copepods have led to an underestimation of copepod grazing.



**Fig 2. A box plot diagram showing inter- and intra-individual differences in gut throughput time (GTT) calculated from 11 individual copepods. Notches on boxes indicate 95% confidence intervals, upper and lower box edges are upper and lower quartiles respectively, whiskers are 1.5x interquartile range, and the red lines inside boxes are the individual median. The black dashed line is the literature value for GTT, based on copepods that had been incubated in filtered seawater. The red dashed line is group median from our experiments. The data show significant differences among individuals, large intra-individual variability, and a lower GTT than the commonly used literature value.**

We deployed our *in situ* imaging system in Dabob Bay to test the feeding sorties vs. *in situ* feeding hypotheses. The feeding sorties hypothesis states that copepods will migrate upwards at dusk to feed in the food-rich euphotic zone, and as soon as they are full, will migrate downwards to digest their food. When they are hungry enough, they migrate upwards again to feed, making multiple sorties to the euphotic zone and back each night. The *in situ* feeding hypothesis states that copepods will migrate upwards at dusk, and stay in the food-rich euphotic zone until dawn, when they will migrate downwards.

To test these hypotheses, we deployed our imaging system 3 times a night; around dusk to intercept the vertical migration of copepods, during the middle of the night to look at the variability of in feeding status, and around dawn to intercept downward migration. The images allowed us to distinguish between copepods with food in their guts, and those that were empty. If the copepods were behaving according to the feeding sorties hypothesis, we predicted that we would see a pulse of empty copepods rising toward the surface. When they reached the phytoplankton-rich layer, they would fill, and very quickly we would observe full copepods at depth (below the food-rich layer). If the copepods were behaving according to the *in situ* feeding hypothesis, we predicted a rising pulse of empty copepods at dusk. These copepods would begin to fill in the phytoplankton-rich layer, and stay there. There would be no layer of full copepods below the food-rich layer.

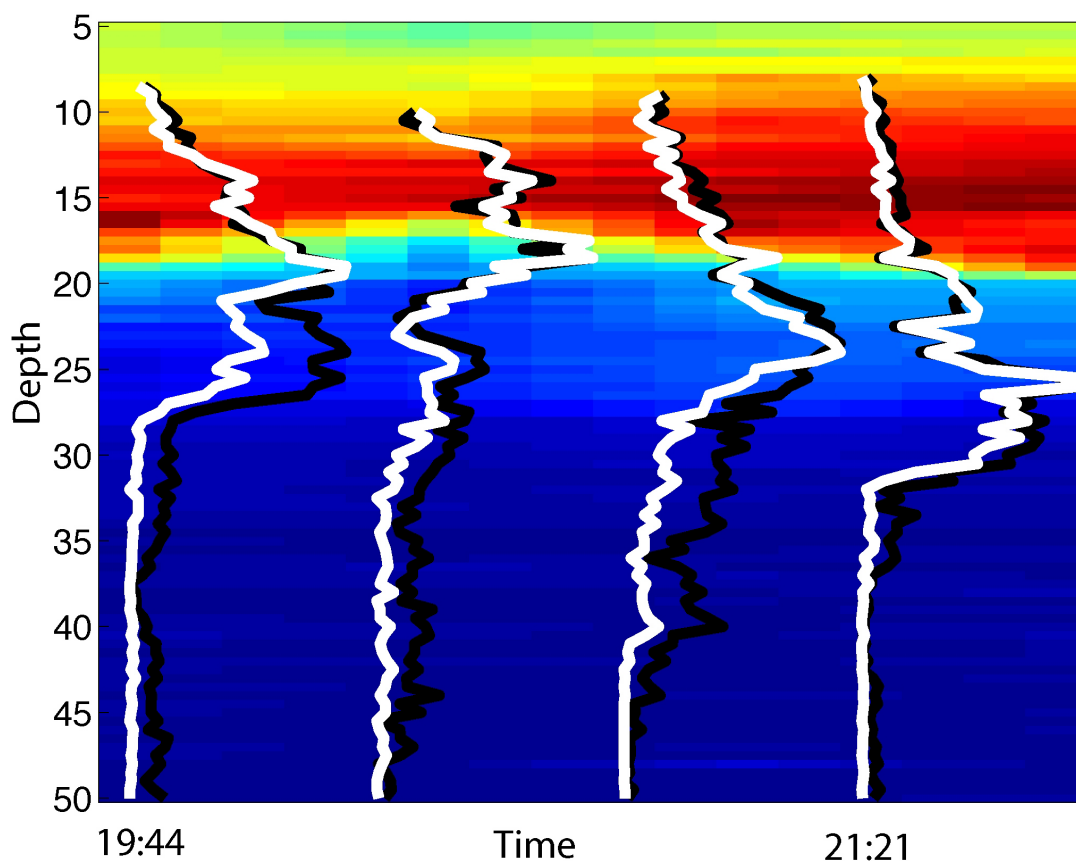
Our data appear to confirm the feeding sorties hypothesis (Fig. 3). Within an hour after their initial ascent, a subsurface maximum in the percentage of fed copepods can be observed. These copepods could not have fed at this depth, as there was too little phytoplankton. The only source of fed copepods at depth had to have been from copepods migrating downward with full guts, only 10's of minutes after eating. These results are quite exciting, as they provide significant new insights into the feeding behaviors of copepods, and the coupling of primary and secondary production in the ocean.

## **IMPACT/APPLICATIONS**

Our bi-spectral PLIF system gives us an entirely new way to gather data from planktonic organisms in the lab and *in situ*. Combined with appropriate auxiliary data, this system will allow us to investigate the dynamics of the planktonic ecosystem at the level of the individual plankters. The data generated will give us a unique and powerful new view into the dynamics structuring marine planktonic ecosystems.

## **RELATED PROJECTS**

This work grew from our ONR-sponsored project entitled “Biological and Chemical Microstructure in Coastal Areas” in which we deployed a PLIF system in tandem with an optical nitrate sensor and microstructure sensor. Based on the information gathered in the present work, we will re-analyze the images acquired in our earlier cruises to attempt to identify zooplankton gut fluorescence in the images.



*Fig.3. Four profiles from a dusk deployment of the bispectral imaging system in Dabob Bay. Black lines are the percent of copepods (relative to the total in the profile) found at a given depth. White lines are the percent of copepods at a given depth that have food in their guts. The background color indicates the phytoplankton fluorescence. At dusk (19:44) the fed copepods are concentrated near the base of the fluorescence maximum; below that is a maximum of unfed copepods. By 21:21 the fed copepods have migrated downward to form a maximum below the fluorescence maximum, rather than staying within the fluorescence maximum. This behavior is consistent with the foraging sorties hypothesis.*

## PUBLICATIONS

Karaköylü, E., P.J.S. Franks, Y. Tanaka, P.L.D. Roberts and J.S. Jaffe. Copepod feeding quantified by planar laser imaging of gut fluorescence. *Limnology and Oceanography Methods*. Accepted.

## HONORS/AWARDS/PRIZES

P.J.S. Franks: Dave Chapman Lecture, WHOI, 2008.